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| MARVICH, MARIA | |

| ART UNIT | PAPER NUMBER |
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| 1633 | |

DATE MAILED: 01/12/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/897,988 | NAKAI, YUTA | |
| | Examiner | Art Unit | |
| | Maria B. Marvich, PhD | 1633 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *07 November 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-3,6,7 and 10* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3,6,7 and 10* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *11 August 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☒ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

This office action is in response to an After-Final Amendment filed 11/7/05. **The amendment has been entered.** Claims 4, 5, 8 and 9 have been cancelled. Claims 1 and 10 have been amended. Claims 1-3, 6, 7 and 10 are pending.

### *Response to Amendment*

Upon further review of the instant claims and specification it is apparent that the application is not in condition for allowance. Therefore, prosecution is reopened. As new grounds of rejection are presented in this action that are not necessitated by applicants' amendment of the claims, this action is non-final.

The instant claims are drawn to a method comprising culturing a microorganism in a medium to produce and cause accumulation of a nucleic acid or L-amino acid in the medium. The microorganism is said to be constructed from *Escherichia* or *Coryneform* bacteria with enhanced high and/or deficient low-energy efficiency enzymes of the respiratory chain pathway. In traversal of art (Ciccognani et al) teaching cultured *E. coli* cells with the recited characteristics, applicants have argued in the amendment filed 1/9/03 that the methods of Ciccognani et al differ from that of the instant invention by accumulation of target substances within the microorganism itself and not in the medium. Applicants' arguments imply some step within the instant method or some characteristic of the instant cell that is distinguishable from Ciccognani et al. However, no such step or characteristic is found in the claims or disclosure that would be demonstrate such a distinction. Applicants demonstrate use of *E. coli* strains transformed with *cyoABCD* or made deficient by deletion of *ndh* that are capable if increased L-

amino acid production. Neither the cells nor steps within the method comprise additional components that are distinguishable from the prior art by leading to accumulation of the amino acids outside of the cell. In fact, it is not apparent that the L-amino acids were isolated from the medium as no explicit step teaching this is found in the specification. Therefore, absent evidence to the contrary, the step of culturing the microorganisms causes the accumulation of the target substance in the media. Furthermore, upon reconsideration the claim language does not explicitly exclude accumulation of nucleic acids and amino acids with in the cells that are part of the medium. Therefore, during normal growth and replication processes nucleic acid and L-amino acids would naturally accumulate within the cell and natural accumulation of the target substances outside of the cell would occur through cellular lysis or rupture or through native excretion pathways and this

### *Claim Objections*

Claim 1 is objected to because of the following informalities: as recited, the Markush listing of enzymes (i.e. SoxM type oxidase, bc1 complex) appears to describe the "energy of efficiency" by directly following this term and not the enzymes themselves. It would be remedial to recite that the enzymes are "selected from the group consisting of".

### *Claim Rejections - 35 USC § 112*

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-3, 6, 7 and 10 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-3 are vague and indefinite in that the metes and bounds of "high-energy efficiency" and "low-energy efficiency" are unclear. The specification defines respiratory chain pathways of high and low energy efficiency as pathways with high and low proton transfer value per election. The terms "high" and "low" are relative terms not defined by the claim, no single set of conditions is recognized by the art as being "high" or "low" and because the specification does not provide a standard for ascertaining the requisite degree, the metes and bounds of this claim cannot be established. **This is a new rejection.**

Claim 1 is vague and indefinite in that the metes and bounds of "mutant strain or genetic recombinant strain" are unclear. It is unclear if by "a mutant or genetic recombinant strain" applicants refer to the parent strain or the strain that is subject to enhancements and /or deficiencies. **This is a new rejection.**

### *Claim Rejections - 35 USC § 112, first paragraph*

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-3, 6, 7 and 10 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of culturing microorganisms to produce and cause accumulation in the medium of L-amino acids or a nucleic acid using *E. coli* strains

transformed with *E. coli cyoABCD,* that encodes cytochrome bo oxidase or made deficient by deletion of endogenous *ndh* that encodes NDH-II, does not reasonably provide enablement for a method of culturing microorganisms to produce and cause accumulation in the medium of L-amino acids or a nucleic acid using any microorganism belonging to the genus *Escherichia* or *Coryneform* in which any activity of a Sox M type oxidase, bc1 complex, cytochrome bo-type oxidase and NDH-I is enhanced and/or cytochrome bd type oxidase or NDH-II is deficient. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims. **This is a new rejection.**

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based on a single factor but is rather a conclusion reached by weighing many factors (See *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter, 1986) and In *re Wands*, 8USPQ2d 1400 (Fed. Cir. 1988); these factors include the following:

**1) Nature of invention**. The instant invention is drawn to methods of L-amino acid and nucleic acid biosynthesis that are improved by increasing energy supply utilized by the host cell during production of these target substances. Hence, the object of the invention is to construct a microorganism showing improved energy efficiency for the production of target substances.

**2) Scope of the invention**. To this end, applicants claim use of a microorganism "constructed from a parent strain belonging to the genus *Escherichia* or *Coryneform*" that "is a

mutant or genetic recombination strain" in which the activity of a high-energy efficiency respiratory chain pathway, i.e. *SoxM* type oxidase, bc1 complex, cytochrome bo-type oxidase and NDH-I is enhanced and/or the activity of a low energy efficiency respiratory chain pathway, i.e. cytochrome bd-type oxidase or NDH-II is deficient. Dependent claims 2 and 3 narrow this genus by recitation that the activity of a high-energy efficiency respiratory chain pathway is enhanced by increasing a copy number of a gene coding for said enzymes or by modifying an expression regulatory sequence of the gene and the activity of a low-energy efficiency respiratory chain pathway is made deficient by disruption of a gene coding for said enzyme. However, as broadly recited in claim 1, enhanced levels of high-energy efficiency enzymes encompasses a broad and diverse genus of conditions such as culturing conditions or stimulation conditions to which the strain is subjected (page 2, paragraph 2, instant specification). Deficiency in production of any of the low energy efficient enzymes encompasses a broad range of conditions such as growth arrest or natural deletion or mutation in the genes. Similarly, modifying an expression regulatory sequence of the gene encompasses a broad range of conditions including repression or activation or substitution. It is noted that the claims recite that the microorganism be a mutant or genetic recombination strain but as recited, the mutation or recombination need not necessarily but can also be responsible for the enhancement or deficiency of activity of the high or low energy efficiency enzymes. The broad scope of the microorganism and methods of altering enzyme activity exacerbates the unpredictable art of metabolic engineering of cells for biosynthetic purposes.

**3) Number of working examples and guidance**. The specification teaches that *E. coli* and *Coryneform* microorganisms comprise a plurality of respiratory chain electron transfer

pathways that function in parallel, which include pathways with high and low proton transfer value per election. High-energy efficiency pathways are said to comprise enzymes involved in the generation of a proton concentration gradient between the inside and the outside of the cytoplasmic membrane with high proton excretion ability and low-energy efficiency pathways describe enzymes with low proton excretion ability. Applicants provide describe the respiratory pathways of *Escherichia coli* and *Corynebacterium glutamicum* in terms of their correlative high and low energy efficiency enzymes (page 2, paragraph 2-3). Specifically, *Escherichia coli* comprises parallel high and low energy efficiency dehydrogenases; NDH-I encoded by the *muo* operon and NDH-II encoded by the *ndh* operon, respectively and comprises high and low energy efficiency oxidases; cytochrome bo-type oxidases encoded by the *cyoABCD* operon (classified as a Sox M type oxidase) and cytochrome bd-type oxidases encoded by the *cydAB,* respectively. *Corynebacterium glutamicum* is said to comprise cytochrome bcl-type oxidases and Sox M-type oxidases (high-energy efficiency) and cytochrome bd-type oxidase (low-energy efficiency). As guidance for enhancing expression, applicants teach that the sequence of the *cyo* operon of *E. coli* (cyoABCD) is known and can be cloned for over-expression in a cell and further propose mutagenesis of the coding sequences of the enzymes to lead to increased intracellular activity. As guidance for deficiency of the low energy pathway, applicants teach that the *ndh* gene of *E. coli* can be cloned and mutated and reinserted into the chromosome to disrupt the gene.

The specification teaches that the microorganism can be any so long as it is imparted these aforementioned properties (see e.g. page 13, paragraph 2) but specifically propose use of strains known in the art i.e. *E. coli* strains W3110(*tyrA*), AJAJ12604 and VBPM B-3996 each of which has been previously engineered for enhanced production of lysine, phenylalanine and

threonine respectively. Specifically, to generate the microorganisms of the instant invention, the *cyoABCD* operon of *E. coli* is introduced into cells resulting in enhanced activity of this oxidase and endogenous NDH-II is deleted. For production of L-lysine, W3110(*tyrA*) transformed with *cyo* or deleted of *ndh* produced 0.48 g/L of L-lysine 0.53 respectively compared to W3110(*tyrA*) which produced 0.29. For production of L-threonine, *E. coli* VKPM B-3996 was transformed with *cyo* and shown to produce 14.3 g/L compared to VBPM B-3996 which produced 13.1. For production of phenylalanine, *cyo* was introduced into W3110(tyrA)/pACMAB, pBR-aroG4 for production of 4.2 g/L compared to non-transformed cells which produced 3.9 g/L. Hence, applicants have demonstrated that they can improve strains previously established for biosynthesis by further manipulation of *ndh* and *cyoABCD* to enhance biosynthetic processes of L-amino acid.

**4) State of the art**. The art of respiration is a complex field. In aerobic respiratory pathway of microorganisms electrons are transferred from electron donors such as NADH dehydrogenase to quinone and then to terminal oxidases. The terminal oxidases constitute a large superfamily that can be classified into three groups based upon homology- SoxM type, Sox B type or FixN oxidases (see Castresana, page 150, col 2, paragraph 2). Cytochrome bd is a terminal oxidase that is widely distributed among archae and bacteria and is present with one more cytochromes (see Castresana, page 150, col 2, paragraph 3). The art teaches that aerobic respiration has a single origin but despite this lack close phylogenetic relationship among present day aerobic organisms (see Castresana and Saraste, page 445, col 2, paragraph 2). Phylogenetic analysis revealed that the distribution of enzymes involved in respiration is very irregular and shows diverse strategies of energy conservation used by prokaryotes. Botts and Niebisch et al,

which review current knowledge of the *C. glutamicum* respiratory chain, teach that there are multiple enzymes involved in respiration of *C. glutamicum*. *C. glutamicum* encodes several electron donors that are depicted in figure 1. Among the electron donors is NDH-II encoded by an *ndh* gene. Botts and Niebisch teach that the *ndh* gene shows 27% similarity to that of *E. coli*. Knock-out experiments have demonstrated that *ndh* is the sole NADH dehydrogenase (page 132, col 2) as well the genome contains an *ndh* gene but not a *nuo* operon. Therefore, the NADH dehydrogenase pathway of *C. glutamicum* does not have a high and low energy efficiency pathway. It is not clear if any of the other electron donors contain high and low energy efficiency pathway enzymes as defined by the instant specification or not as the art is silent as to the high or low energy efficiency of these enzymes and the terms high and low are relative such that a person of skill in the art would not be able to ascribe specific meaning to them. *C. glutamicum* contains at least two terminal oxidases, cytochrome bc1 and cytochrome bd, also depicted in figure 1, which according to the specification are members of the high and low energy efficiency pathways, respectively.

The art of metabolic engineering is highly unpredictable exacerbating the unpredictable nature of the instant invention. Bailey (Science, June 1991) teaches that metabolic engineering "enables construction of metabolic configurations with novel and often beneficial characteristics" but "at present, metabolic engineering is more a collection of examples than a codified science" (page 1668, column 1, paragraph 2 and 3). Furthermore, he teaches "Many studies have shown the feasibility of metabolic engineering methods without achieving the yields, rates or titers (final concentrations) required for practical processes." (page 1668, column 1, paragraph 3). The obstacles Bailey details are that the cell has complex cellular responses to

genetic perturbations that complicate predictive design. The complex cellular responses include an inability to anticipate further reactions which leads to iterative cycles of genetic modifications, an inability to predict metabolic consequences following the transfer of heterologous genes into the cell as well as rearrangements and deletions of chromosomal and plasmid DNA.

More recently, Parekh et al. (Appl Microbiol Biotechnol, 2000) teach that "...heterologous protein expression in bacterial or fungal systems offers a significantly complex ,metabolic network. The rate-limiting enzymatic reactions or flux nodes are unknown in most if not all pathways and probably change with each new culture. Thus with limited knowledge of the physiology and genetics associated with the production of each molecule of interest one is often led to an empirical approach to strain improvement. (page 288, column 1, paragraph 1)." Further problems are encountered with the need to scale-up processes such as false positives are encountered and an inability to maintain the same physical environment (page 299, column 1, paragraph 2-3).

**5) Unpredictability of the art**. The MPEP teaches, "However, claims reading on significant numbers of inoperative embodiments would render claims non-enabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining those that are operative. Atlas Powder Co. v. E.I. duPont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); In re Cook, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971). (see MPEP 2164.08(b).

The instant method requires as an essential element, alteration of the respiratory chain pathway such that the activity of a high-energy efficiency enzyme is enhanced and/or the activity

of a low-energy efficiency enzyme is deficient. It is applicants' goal to improve energy utilization of the strain as a means to enhance L-amino acid production. As guidance, the specification teaches that overexpression of homologous genes or deletion of endogenous genes can generate a strain showing improved energy efficiency. And specifically, applicants demonstrate that *E. coli* strains transformed with *cyoABCD* or made deficient by deletion of *ndh* do lead to enhanced production of L-amino acids. However, the correlative enhancements or deficiencies to generate the recited strains are unclear given the broad nature of the recited alterations. Means of enhancing the activity or making deficient the activity of an enzyme encompasses a broad and diverse group of conditions that include growth conditions or stimulation conditions as well as mutations or recombinations. For example, the specification teaches that the levels of expression of the respiratory chain enzymes vary in response to their growth environment (see page 2, paragraph 2). Similarly, deficiency in production of any of the low-energy efficiency enzymes encompasses a broad range of conditions such as growth arrest or natural deletion or mutation in the genes. While, applicants recite that the high energy efficiency enzymes can be enhanced by modification of the expression regulatory sequences of the genes, applicants have not demonstrated what types of modifications would result in the enhancements. Determining those enhancements, deficiencies, modifications, mutations or genetic recombinations that would generate a strain with improved energy utilization would require undue experimentation.

While applicants have demonstrated that *E. coli* can be engineered for enhanced amino acid production by overexpression of *cyoABCD* or made deficient by deletion of *ndh*, it is unpredictable that correlative enzymes for the recited high-energy efficiency enzymes (SoxM

type oxidases, bc1 complexes, cytochrome bo-type oxidases, NDH-I) and the low-energy efficiency (cytochrome bd-type oxidase or NDH-II) can be identified for the entire genus of *Escherichia* or *Coryneform* such that the recited strains are produced. First, applicants recite that the organism must have enzymes of high and low energy efficiency. However, it is not clear what levels of efficiency are required for an enzyme to be of high or low efficiency as these are relative terms. The terms high and low-energy efficiency enzyme does not provide the structural or functional requirements that would allow adequate identification of correlative enzymes. Secondly, the art of discernment of energetic efficiency is a contested topic and as such is an unpredictable art hence identifying genes other than those that encode NDH-I, NDH-II, cytochrome bd and cytochrome bo is not guaranteed as the means to determine the efficiency of energy of the proteins these genes encode. As taught by Neijssel and de Mattos (Molecular Microbiology (1994) 13(2), 179-182), "the efficiency of energy conservation by bacterial respiratory chains using intact cells could not (and still cannot) be determined as simply as in mitochondria and cell-free preparations yielded unreasonably low P/O ratios" (page 180, column 1, paragraph 1). They further teach "it can be shown that there are many energy-spilling reactions in the cell whose physiological functions are sometimes unclear (e.g. futile cycles), but whose activity *in vivo* may well vary with growth rate. It is extremely difficult if not impossible to determine the activities of these reactions in growing cultures, and this implies that one cannot derive a reliable estimate of the efficiency of the respiratory chain from measurements of growth yields" page 180, column 2, paragraph 2).

Hence applicants have only demonstrated that for *E. coli* transformed with *E. coli cyoABCD,* that encodes cytochrome bo oxidase or made deficient by deletion of endogenous *ndh* that encodes NDH-II, enhanced production of amino acids can occur.

**6) Amount of Experimentation Required**. The invention recites a method of producing and causing accumulation of target substances in the medium using constructed microorganisms. Given the lack of guidance in the specification, the large and diverse group of microorganism and enzymes to be altered as well as broadly recited means of altering their activity and the highly unpredictable nature of the art, it is concluded that a person of skill in the art would have had to conduct undue experimentation in order to practice the claimed invention.

Claims 1, 6, 7 and 10 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. **This is a new rejection.**

In the instant case, applicants recite use of a genus of "mutant or genetic recombinant" *Escherichia* or *Coryneform* microorganisms in which the activity of an enzyme from the respiratory pathway of high-energy efficiency is deficient and/or activity of an enzyme from the respiratory pathway of low-energy efficiency is deficient. As such, applicants' claims are drawn to a broad and diverse genus of microorganism coupled with a broad and diverse genus of methods of enhancing and/or making deficient the enzymes of the respiratory pathway for use in the method of producing and causing accumulation of target substance in the medium.

The written description requirement for genus claims may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlations between function and structure, or by a combination of such characteristics sufficient to show that the applicant was in possession of the claimed genus.

Although the instant claims are directed to methods, adequate description of the methods first requires an adequate description of the materials, which provide the means for practicing the invention. The Guidelines for Written Description state "The claimed invention as a whole may not be adequately described if the claims require an essential or critical element which is not adequately described in the specification and which is not conventional in the art". As an essential element of the invention, applicants have disclosed that strains are generated with improved energy utilization to improve methods of amino acid and nucleic acid biosynthesis. Applicants have demonstrated use of *E. coli* strains transformed with *E. coli cyoABCD* that encodes cytochrome bo or made deficient by deletion of endogenous *ndh* that encodes NDH-II in methods of production of amino acids. By this disclosure, applicants have not demonstrated that they are in possession of the large genus of recited strains given the broad and diverse nature of the strains.

First, by recitation that "the activity of the enzyme" is enhanced and/or made deficient, applicants recite a broad and diverse set of conditions. Enzyme activity can be enhanced i.e. modifying the regulatory sequence of said gene or made deficient by a broad and diverse set of conditions that include growth conditions or stimulation conditions as well as mutations or

recombination. For example, the specification teaches that the levels of expression of the respiratory chain enzymes vary in response to their growth environment (see page 2, paragraph 2). Similarly, deficiency in production of any of the low energy efficient enzymes encompasses a broad range of conditions such as growth arrest or natural deletion or mutation in the genes. As well regulatory sequences can be modified by treatment with compounds or by mutation. Applicants have only demonstrated construction of strains overproducing *cyo* strains and deficient NDH-II strains. By disclosure of the two alterations, applicants have not described the broad genus of enhancements or deficiencies such that a person of skill in the art would be able to envision any alterations for enhancement or deficient levels or modifications of the recited enzymes or regulatory sequences such that strains with improved energy utilization are generated.

Secondly, applicants recite that the enzymes are members of high and low energy efficiency pathways of the respiratory chain of *Escherichia* or *Coryneform.* Hence, applicants recite a broad genus of enzymes for which the structural and functional requirements are unclear. Functionally, the enzymes must be high and low energy efficiency enzymes. However, it is not clear what levels of efficiency are required for an enzyme to be of high or low efficiency as these are relative terms. The terms high and low-energy efficiency enzyme does not provide the structural or functional requirements that would allow adequate identification of correlative enzymes. As well, the art of determining the energy efficiency of an enzyme is a contested and unpredictable topic. Hence, it is unpredictable the correlative enzymes can be identified for the entire genus of *Escherichia* or *Coryneform* based upon these functional requirements. For example, the art teaches *C. glutamicum* encodes cytochrome bc1, cytochrome bd and NDH-II but

does not encode NDH-I and it is not clear what other of the dehydrogenases comprises high and low efficiency pathways. From the specification, it appears that cytochrome bc1, cytochrome bd would be considered enzymes of the high and low efficiency pathway. Furthermore, the specification and the prior art has not established a strong correlation between the structure of the strains and their function in improving energy utilization and one skilled in the art cannot predict with a reasonable degree of confidence the structure of the claimed invention from the recitation of its function. Therefore, there is no clear description of the compositional (i.e. "structural") or functional characteristics required of the enzymes for the variety of cell types intended for use in the instant invention. By disclosure of *E. coli* strains transformed with *E. coli cyoABCD,* that encodes cytochrome bo oxidase or made deficient by deletion of endogenous *ndh* that encodes NDH-II, applicants have only demonstrated two species. For inventions in an unpredictable art, adequate written description of a genus cannot usually be achieved by disclosing only one or two species within the genus. Therefore, the skilled artisan cannot conclude that the applicant was in possession of the claimed invention. Given the widely divergent nature of enhancements, deficiencies and modifications and the broad and diverse nature of the recited enzymes and the uncertainty of the combinations of alterations and enzymes to generate strains with improved energy utilization, it must be considered that any strain must be empirically determined.

***Claim Rejections - 35 USC § 102***

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-3, 7 and 10 are rejected under 35 U.S.C. 102(b) as being anticipated by Ciccognani et al (FEMS Microbiology Letters 94, 1992, page 1-6; see entire document). **Upon reconsideration, this rejection made in the office action mailed 8/9/02 is reinstated. The rejection is restated below.**

Ciccognani et al teach methods of culturing *E. coli* (RG145), which according to the instant specification comprise a high and low-energy efficiency respiratory chain pathway. RG145 is a genetic recombinant strain in which an enzyme of the high-energy efficiency pathway was enhanced and an enzyme of low-energy efficiency was deficient. The cells within the media comprise nucleic acid or L-amino acid and were collected resulting in collection of the nucleic acid and L-amino acid as recited in claims 1 and 10. The cells contain a chromosomal deletion resulting in the inability of the cell to express *cydA* and contain a cosmid containing the *cyo* operon resulting in over expression of the cytochrome bd complex (page 2, section 3.1) as recited in claims 2 and 3

Claims 1, 2, 7 and 10 are rejected under 35 U.S.C. 102(a) as being anticipated by Spehr et al (Biochemistry, 1999, Vol 38, pages 16261-16267; see entire document). **This is a new rejection.**

Spehr et al teach methods of culturing *E. coli* cells (ANN003/pAR1219), which according to the art and the instant specification comprise a high and low-energy efficiency

respiratory chain pathway. ANN003/pAR1219 is a genetic recombinant strain in which an enzyme of the high-energy efficiency pathway was enhanced. The cells within the media comprise nucleic acid or L-amino acid and were collected resulting in collection of the nucleic acid and L-amino acid as recited in claims 1 and 10. The *nuo* operon was cloned and expressed under control of the inducible T7Φ10 promoter in *E. coli* cells for overexpression as recited in claim 2.

***Response to Argument***

Applicants traverse the claim rejections under 35 U.S.C. 102 on pages 4-5 of the amendment filed 1/9/03. Applicants argue that Ciccognani et al disclose production of cytochrome within the microorganism itself and not in the medium in which the microorganism is cultured. Furthermore, applicants argue that by teaching that the target substance accumulates in the microorganism, Ciccognani et al teach away from the instant invention and fail to teach or suggest the instant invention.

Applicants' arguments filed 1/9/03 have been fully considered but they are not persuasive. Applicants' arguments imply some step within the method or provided by the cell in the instant invention that is distinguishable from Ciccognani et al. However, no such step or characteristic is found in the claims or disclosure that would be demonstrate such a distinction. Applicants demonstrate use of *E. coli* strains W3110(*tyrA*), AJ12604 and VBPM B-3996 transformed with *cyoABCD* or made deficient by deletion of *ndh* were used to produce lysine, phenylalanine and threonine, respectively. Neither the cells nor steps within the method comprise additional components that are distinguishable from the prior art by leading to

accumulation of the amino acids outside of the cell. In fact, it is not apparent that the amino acids were isolated from the medium as no explicit step teaching this is found in the specification. A review of the art teaches that "Microbial cells typically have mechanisms for transporting amino acids into the cell, but the mechanisms of export of high levels of amino acids is not known in many cases" (see paragraph 635, McFarlan et al). In fact, for biosynthetic purposes, metabolic engineering methods have been used to develop strains that excrete amino acids as means of enhancing production (see Srinivasan and Choi, col 2, line 56-64 and Bachmann et al, col 1, line 19-35). Srinivasan and Choi teach methods of mutating or constructing by genetic recombination microorganisms to cause excretion of amino acids outside of the cell. Bachmann et al teach that *Corynebacterium glutamicum* requires plasmids that support excretion of amino acids for excretion (col 1, line 19-35). Therefore, it appears that native strains do not actively excrete target substances such as amino acids into the medium but require specific engineering steps to generate appropriate strains for accumulation of the substances in the medium. However, the instant invention does not teach means of engineering the cells for active excretion. Therefore, it appears as if the step of culturing the microorganisms actually is causes accumulation of the target substance in the media. First, during normal growth and replication processes nucleic acid and L-amino acids would naturally accumulate within the cell and natural accumulation of the target substances outside of the cell would occur through cellular lysis or rupture or through native excretion pathways and this Furthermore, upon reconsideration the claim language does not explicitly exclude accumulation of nucleic acids and amino acids with in the cells that are part of the medium.

***Conclusion***

Claims 1-3, 6, 7 and 10 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria B. Marvich, PhD whose telephone number is (571)-272-0774. The examiner can normally be reached on M-F (6:30-3:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, David Nguyen, PhD can be reached on (571)-272-0731. The fax phone numbers for the organization where this application or proceeding is assigned are (571) 273-8300 for regular communications and (571) 273-8300 for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Maria B Marvich, PhD
Examiner
Art Unit 1633

January 9, 2006